

AMENDMENT & RESPONSE UNDER 37 C.F.R. § 1.116 - EXPEDITED PROCEDURE

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Title: NON-TOXIC MUTANTS OF PATHOGENIC GRAM-NEGATIVE BACTERIA

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substantially reduced toxicity when compared to the endotoxin of the wild type gram-negative bacterial pathogen, and

purifying the mutant endotoxin from the *htrB* mutant pathogen.

E2
Sub 22

23. A mutant endotoxin made according to the method of claim 22, wherein the mutant endotoxin was purified from the *htrB* mutant pathogen by phenol-water extraction or by protease digestion.

24. The mutant endotoxin according to claim 23, wherein the mutant endotoxin is conjugated to a carrier protein.

25. A mutant endotoxin made according to the method of claim 22.

26. The mutant endotoxin according to claim 25, wherein the mutant endotoxin is conjugated to a carrier protein.

Sub 22
E3

29. [Amended] A method for producing endotoxin-specific antisera, the method comprising

(a) immunizing an individual with a vaccine formulation comprising an *htrB* mutant of a gram-negative bacterial pathogen, endotoxin isolated from the *htrB* mutant of the gram-negative bacterial pathogen, or endotoxin purified from the *htrB* mutant of the gram-negative bacterial pathogen wherein the endotoxin is conjugated to a carrier protein; and

(b) collecting antibody produced from the immunized individual;

wherein the *htrB* mutant lacks one or more secondary acyl chains of lipid A contained in a wild type gram-negative bacterial pathogen and lacks 3-hydroxy unsaturated C16 fatty acid substitutions on the lipid A as compared to a wild-type bacterial pathogen resulting in substantially reduced toxicity when compared to lipid A of the wild type gram-negative bacterial pathogen.